Analytical evaluation of a rapid on-site oral fluid drug test

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ABSTRACT

There is a need for a reliable rapid on-site oral fluid test that can be used in police controls to detect impaired drivers.

We evaluated the Varian Oralab®6 and collected two oral fluid samples from 250 subjects, one with the Varian Oralab®6 and one with the StatSure™ Saliva•Sampler™.

The Oralab®6 can detect six drug types: amphetamines, methamphetamine, cocaine, opiates, delta9-tetrahydrocannabinol (THC) and phencyclidine (PCP). On-site results were obtained within 10 to 15 minutes. The sample collected with StatSure™ was analysed using Liquid Chromatography – tandem mass spectrometry after liquid-liquid extraction and these results were used as a reference to determine prevalence, sensitivity and specificity.

Two cut-off values were used in the evaluation. The Varian cut-off values were: amphetamine 50 ng/mL, cocaine 20 ng/mL, opiates 40 ng/mL and THC 50 ng/mL. The DRUID cut-offs were: amphetamine 25 ng/mL, cocaine 20 ng/mL, opiates 20 ng/mL and THC 1 ng/mL. Applying the first cut-offs, prevalence, sensitivity and specificity were: amphetamine: 10%, 76%, 100%; cocaine 23%, 34%, 100%; opiates 38%, 83%, 94% and THC 18%, 41%, 99%. The DRUID cut-off values gave the following results: amphetamine: 14%, 56%, 100%; cocaine 28%, 34%, 100%; opiates 49%, 68%, 98% and THC 45%, 16%, 99%.

The specificity of the Oralab®6 is generally good. For both cut-offs, sensitivity was low for cocaine and THC. Therefore, the Varian Oralab®6 test is not sensitive enough to be applied during roadside police controls.

Keywords: saliva – point of care test - Varian Oralab®6 – sensitivity

Article outline

Introduction

Oral fluid is known as a suitable matrix for the detection of drugs [1]. The presence of several drugs in oral fluid correlates relatively well with impairment and it has several advantages compared to the use of urine or blood for the detection of drugs [2]. These characteristics led to the search for a rapid on-site oral fluid test that can be used in police controls to detect impaired drivers. In Belgium, France and Australia, legislation permits use of such oral fluid tests during police controls [3].

In several studies different oral fluid tests have been evaluated, mostly with disappointing results. The Roadside Testing Assessment-2 project (Rosita-2) evaluated nine rapid on-site oral fluid tests analytically, among which the previous Varian OraLab, from 2003 till 2005. None of these tests were found to be sensitive enough to be used on a large scale [4]. Later evaluation of several other tests, for example the Dräger DrugTest® [5], the Cozart® RapiScan System [6] and the Drugwipe 5 [7], could not show significant improvement.

The aim of this study was to determine the reliability of the On-site® Varian OraLab®6 (Varian Inc., Lake Forest, California, USA) for detection of drugs of abuse. Therefore, sensitivity and specificity were determined based on the results of 250 collected oral fluid samples with the Oralab®6, compared to the results of a second oral fluid sample obtained as reference, analysed with ultra pressure liquid chromatography – tandem mass spectrometry (UPLC-MS/MS).

This study was conducted within a project financed by the European Commission, 'Driving Under the Influence of Drugs, Alcohol and Medicines' (DRUID). This project was set up to investigate the influence of psychoactive substances – drugs, alcohol and medicines – on traffic safety. Within the work package 'enforcement', several on-site oral fluid tests, among which the Varian Oralab, are evaluated, from an operational and analytical perspective [8].

Method and materials

Samples

Oral fluid samples were collected from 250 subjects. Two hundred were obtained in a rehabilitation centre for drug addicts, the other 50 subjects were selected during roadside surveys. Two oral fluid samples were collected, one with the Varian Oralab®6, and the other with the StatSure™ Saliva•Sampler™ (StatSure™, Brooklyn, NY, USA), an oral fluid collector.

Varian OraLab instrument

The Oralab®6 test consists of 3 elements: an oral fluid collector, the test tube and the test card, as shown in Figure 1. The oral fluid collector is a swab with a salty taste (to increase saliva production), the test tube contains the oral fluid when it is squeezed out of the swab and it is shaped to hold the test card. The test card is the actual lateral-flow immunoassay to detect drugs in the oral fluid.

Since this study, the Varian Oralab®6+ has been introduced, it differs in design compared to the Varian Oralab®6, but the immunoassay itself is still the same.

Figure 1: The Varian Oralab®6 on-site fluid test, consisting of an oral fluid collector, the test tube and the test card (inside the tube)

Oral Fluid Collector

The oral fluid for the sample with the Oralab®6 was collected by keeping the swab under the tongue for about three minutes, which normally was sufficient to collect a minimum of 1mL oral fluid and proceed with the test.

Test tube

After collection of the oral fluid with the swab, the latter was squeezed out in the test tube. The test card was inserted in the tube to perform the test and removed within 15 minutes to read the results. The test tube with the remaining oral fluid was stored in the freezer for later analysis.

Test card

The Oralab®6 detects amphetamine (AMP), methamphetamine (METH), opiates (OPI), cocaine (COC), delta9-tetrahydrocannabinol (THC) and phencyclidine (PCP). The test card is a qualitative immunoassay, based on competitive inhibition. For each of the six tested drugs, immobilized drug conjugates and red-labeled antibody-coated micro particles are attached to a membrane. By introducing the test card into the tube, the membrane of the test card absorbs the oral fluid. The drugs in the sample compete with and exceed the drug conjugates for interaction with the antibody coated micro particles, which results in a clean line next to the first character of the corresponding drug name. Absence of drugs in the sample results in a red line. There are also two control lines to ensure the oral fluid was sufficiently migrated by the test card.

Fig. 2 shows a test card that is positive for opiates and negative for the other drugs.

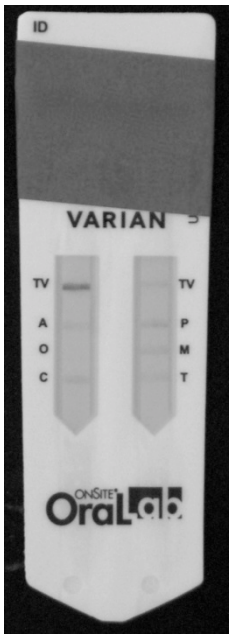


Figure 2: A Varian Oralab®6 test card, positive for opiates (no line) negative for the other drugs. TV: test valid, A: amphetamine, O: opiates, C: cocaine, P: phencyclidine, M: methamphetamine, T: cannabinoids

StatSure™ Saliva•Sampler™

Since there is no buffer in the test tube of the Varian Oralab®6, a good conservation of the drugs, particularly THC, during storage is not guaranteed. There is a risk of drugs adhering to the oral fluid collector or to the test tube. Therefore, a second saliva sample was collected with the StatSure™ Saliva•Sampler™. This is an oral fluid collector with good recovery and stability of drugs in the oral fluid during storage [9]. The concentrations of drugs found in this second sample after analysis with LC-MS/MS were adjusted for volume, based on the weight of the StatSure™ device after collection.

Fig. 3 shows the StatSure™ Saliva•Sampler™, which consists of two elements: an oral fluid collector and a transport tube.

Figure 3: The StatSure™ Saliva•Sampler™, consisting of an oral fluid collector and a transport tube

Oral fluid collector

The StatSure™ oral fluid collector is also a swab that has to be kept under the tongue to absorb saliva. In this case, there is an indicator in the plastic handle that turns blue when 1 mL oral fluid is collected.

Transport tube

The transport tube contains 1 mL of buffer that dilutes the saliva sample and results in a good conservation of the drugs in the sample. The transport tube was stored in the freezer for later analysis with LC-MS/MS.

Standards and reagents

All chemicals and solvents used were analytical or HPLC grade. Ammonium acetate was obtained from Fluka (Bornem, Belgium), Heptane was purchased from Sigma-Aldrich (Bornem, Belgium), ethyl acetate and methanol from Biosolve (Valkenswaard, The Netherlands). Water was purified by an Elga Medica R 7 system from Rossmark (Ede, The Netherlands). All standards and deuterated internal standards were purchased from LGC Promochem (Molsheim France).

Confirmation analysis

Liquid-liquid extraction

The StatSure™ sample was prepared with liquid-liquid extraction before analysis. From the sample, 400 µL was used. Twenty µL of a 20 ng/mL solution of isotope-labeled internal standards was added. Two hundred µL ammonium bicarbonate (0.2 M, pH 9.3) and 1.25 mL heptane/ethyl acetate (1:4) were added. This mixture was vortexed (5 seconds), shaken (15 minutes) and centrifuged (3000 rpm; 5 minutes). The organic phase was removed and evaporated at room temperature. After addition of 100 µL H_2O/methanol (50:50), vortexing (5 seconds) and centrifuging (2 minutes), the sample was transferred to a vial for analysis with UPLC-MS/MS.

Ultra performance liquid chromatography

The Acquity™ Ultra Performance liquid chromatograph (Waters, Zellik, Belgium) was equipped with an Acquity UPLC ® BEH C18 column (1.7 µm; 2.1 x 50 mm), and a Vanguard BEH C18 precolumn (1.7 µm; 2.1 x 5 mm).

As mobile phase a gradient elution of H_2O with 2 mM NH_4HCO_3, pH 9.30 (mobile phase A) and LC-MS methanol (mobile phase B) was applied, as shown in figure 4. The temperature of the column was 60°C, the temperature of the sample manager 10°C and the injection volume was 25 µL.

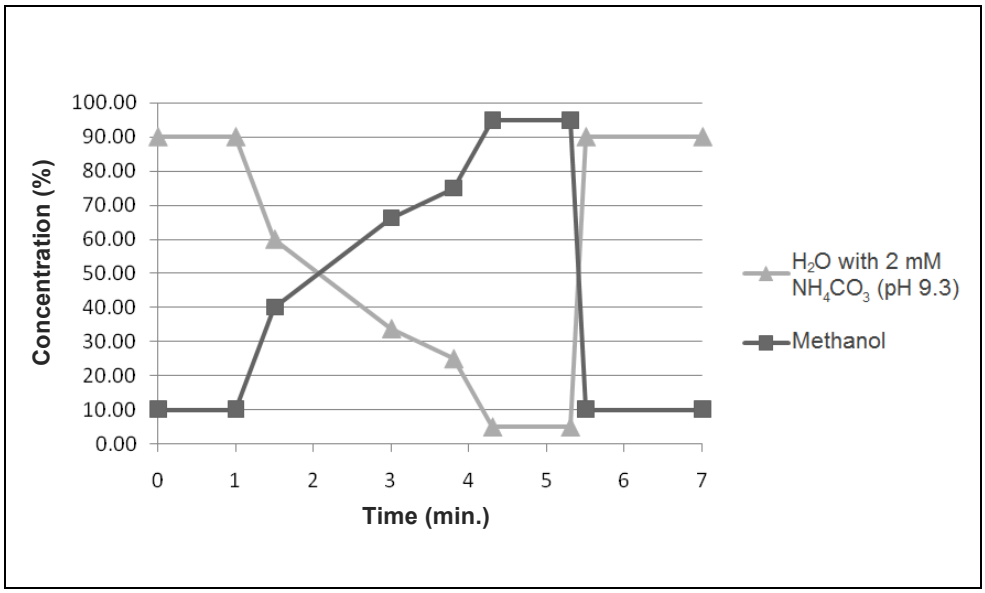


Figure 4: Gradient elution as mobile phase, with mobile phase A (H_2O with 2mM NH_4CO_3) and mobile phase B (methanol)

Tandem mass spectrometry

A Quattro Premier™ XE (Waters) was used, with an Electrospray Ionization-source in positive mode.

The general characteristics of the tandem mass spectrometry are shown in table 1. The different components that were measured and some of their corresponding parameters are summarized in table 2.

~~Imprecision and inaccuracy were lower than 15% for all analytes at the DRUID cut-offs, demonstrating that the limit of quantitation (LOQ) of the method was lower than required by the DRUID consortium.~~

The regression model was quadratic for all components, except for THC, for which the model was linear. The regression model fitted for all analytes within the 0.5-200 ng/mL calibration range (r^2>0.99 for all analytes, except for benzoylecgonine: 0.98). And although absolute matrix effects were observed with some analytes, the relative matrix effect (measured using the coefficient of variation of slopes of standard lines from five different sources) was lower than 2% and hence it can be assumed that the absolute matrix effects observed did not affect the quantification of analytes (Table 3).

Table 1: General parameters for mass spectrometry

	Parameter	**Value**
ES+	Capillary Voltage	0.8 kV
	Extractor Voltage	4 V

	RF Lens	0 V
	Source Temperature	140 °C
	Desolvation Temperature	450 °C
	Desolvation Gas Flow	1000 L/h
	Cone Gas Flow	50 L/h
Analyser	Collision Gas Flow	0.15

Table 2: Measured components with the corresponding parameters

Component	**Q 1**	**Q 3**	**Dwell time (sec)**	**Retention time (min)**	**Cone (Volt)**	**Collision Energy (eV)**
6-acetylmorphine	328.12	152.08	0.035	3.61	47	61
6-acetylmorphine-D3	331.10	164.90	0.035	3.58	45	37
Amphetamine	136.07	119.05	0.035	3.43	15	9
Amphetamine-D5	141.01	92.90	0.035	3.37	17	27
Benzoylecgonine	290.14	168.00	0.035	2.64	33	19
Benzoylecgonine-D3	293.10	171.00	0.035	2.65	33	19
Cocaine	304.11	182.1	0.015	4.66	31	19
Cocaine-D3	307.10	185.00	0.015	4.64	31	19
Codeine	300.14	165.01	0.030	3.85	41	43
Codeine-D3	303.10	215.00	0.030	3.83	45	25
MDA	180.02	105.03	0.035	3.28	15	21
MDA-D5	185.01	110.00	0.035	3.23	17	21
MDEA	208.10	162.97	0.030	3.79	23	13
MDEA-D5	213.07	162.90	0.030	3.69	21	13
MDMA	194.10	162.95	0.035	3.43	21	13
MDMA-D5	199.10	135.20	0.035	3.34	21	21
Methamphetamine	149.96	90.95	0.030	3.61	21	17
Methamphetamine-D5	155.00	120.90	0.030	3.55	19	11
Morphine	286.11	152.10	0.035	3.13	45	53
Morphine-D3	289.08	164.90	0.035	3.10	43	37
THC	315.18	193.05	0.060	5.44	31	21
THC-D3	318.13	196.00	0.020	5.43	31	25

Table 3: validation parameters for the different analytes in the UPLC-MS/MS method.

Component	R^2	Imprecision (%)^	Inaccuracy (%)^	DRUID cut-off (ng/mL)	Extraction yield* (%)	LOQ (ng/mL)	Absolute matrix effect (%)£	Relative matrix effect (CV)$
6-acetylmorphine	0.999	13.6	-3.6	5.0	75.1	5.0	-11.5	1.8
Amphetamine	0.993	6.8	+6.2	25	54.8	10.6	16.2	2.0
Benzoylecgonine	0.983	3.4	+2.4	10	2.8	5.5	8.7	1.8
Cocaine	0.999	8.4	-2.2	10	78.3	6.4	3.1	1.0
Codeine	0.999	7.0	-0.8	20	70.4	0.5	19.7	1.6
MDA	0.998	7.7	+1.5	25	58.1	16.7	1.3	1.8
MDEA	0.997	10.4	-3.5	25	70.3	0.5	11.5	1.7
MDMA	0.997	9.6	+1.8	25	65.9	0.5	4.2	1.5
Methamphetamine	0.997	6.6	+0.4	25	56.5	0.5	6.6	1.4
Morphine	0.997	8.3	+0.5	20	39.5	0.5	6.0	1.1
THC	0.998	8.8	-3.9	1.0	52.6	1.0	93.5	1.1

^ imprecision and inaccuracy determined at DRUID cut-offs [8]; * extraction yield determined at 20 ng/mL;

£ absolute matrix effect determined at 100 ng/mL; $ CV of slopes of standard lines from five different sources

Cut-off values

The cut-off values stated by Varian for the OraLab are 50 ng/mL amphetamine, 50 ng/mL methamphetamine, 20 ng/mL cocaine, 40 ng/mL opiates, 50 ng/mL THC and 10 ng/mL PCP. However, within the Driving Under the Influence of Drugs-project (DRUID) other confirmation cut-off values are determined. These cut-off values are 25 ng/mL amphetamine, 25 ng/mL methamphetamine, 20 ng/mL cocaine, 20 ng/mL opiates and 1 ng/mL THC. For PCP no cut-off value was determined, since this drug is very rarely used in Europe.

The recommended cut-off values as mentioned in *Guidelines for research on drugged driving* [10] are 20 ng/mL for amphetamine and methamphetamine, 10 ng/mL for cocaine, 20 ng/mL for codeine and morphine, 5 ng/mL for 6-acetylmorphine and 2 ng/mL for THC. The DRUID cut-offs are less sensitive for amphetamine, methamphetamine, cocaine and 6-acetylmorphine, but more sensitive for THC.

Cross reactivity

In order to compare the UPLC-MSMS concentrations with the Varian cut-offs, the concentrations were added, taking the cross-reactivity of the OraLab-6 into consideration:

*COC+BE = conc(COC) + 0.067 * conc(BE)*

*OPI = conc(MORPH) + 1.4 * conc(6-AM) + 1.4 * conc(COD)*

Results

Drugs found by UPLC-MS/MS

Table 4 describes the analytical results for the different drugs. Often, very high concentrations were found, suggesting recent drug use.

Table 4: UPLC-MS/MS results for the individual drugs: number of positive samples, lowest, median and highest concentrations observed in the samples where the drug was detected. MDA, MDEA, MDMA and methamphetamine were not observed.

Component	n	Lowest Concentration (ng/mL)	Median Concentration (ng/mL)	Highest Concentration (ng/mL)
6-acetylmorphine	109	5.4	75.4	9787
Amphetamine	33	25.0	685.1	21153
Benzoylecgonine	48	10.9	81.5	14155
Cocaine	54	10.3	52.2	20632
Codeine	71	20.1	60.7	742
Morphine	97	20.6	186.7	9159
THC	112	1.0	31.4	3967

Prevalence, sensitivity, specificity and accuracy

The reliability of the Oralab®6 compared to the results of the StatSure™ sample is given in Table 5. There were no positive results for methamphetamine and PCP gave 5 false positive results with the on-site test.

Table 5: Prevalence, sensitivity, specificity and accuracy of Oralab®6 for DRUID and Varian cut-off values

	DRUID Cut-off					Varian Cut-off*			
	Prev (%)	Sens (%)	Spec (%)	Acc (%)		Prev (%)	Sens (%)	Spec (%)	Acc (%)
COC+BE	22.5	33.9	100	85.1		17.2	37.2	98.5	87.9
OPI	45.0	75.0	97.8	87.6		40.9	82.3	97.9	91.5
THC	44.9	16.0	98.5	61.4		17.6	40.9	99.0	88.7
AMP	13.2	55.6	100	94.3		10.0	76.0	100	97.5

*adjusted for cross-reactivity: COC+BE=COC+0.067 x BE; OPI=MORPH+1.4 x 6-AM+1.4 x COD

To determine the reliability of the Varian Oralab®6, the DRUID cut-off values must be used. The results with the Varian cut-off values, which are higher, were used to determine if the manufacturer could meet up to their own standards.

Applying the DRUID cut-offs, specificity was high for all drugs. Sensitivity was relatively high for opiates, medium for amphetamines and cocaine, and very low for THC. Comparing these results to the ones achieved with the Varian cut-off values, the latter give generally better results, due to the higher detection limits. The biggest differences are seen for THC, but the cut-off values between Varian and DRUID differ 50-fold (respectively 50 ng/mL and 1 ng/mL).

In figures 5 to 8, the log of the StatSure™ concentrations was plotted against the test card results and presented as box-and-whisker plots. The outliers are represented by circles, the extreme outlier by a triangle.
For all drugs, there was a wide spread of drug concentrations that yielded a negative test card result. Moreover, there is an overlap between the negative and positive test card results for THC, cocaine and opiates, which means there was no clear distinction in the concentrations that give a negative result with the oral fluid test and those with a positive result. Only for amphetamine there was a clear distinction, except for the outliers.

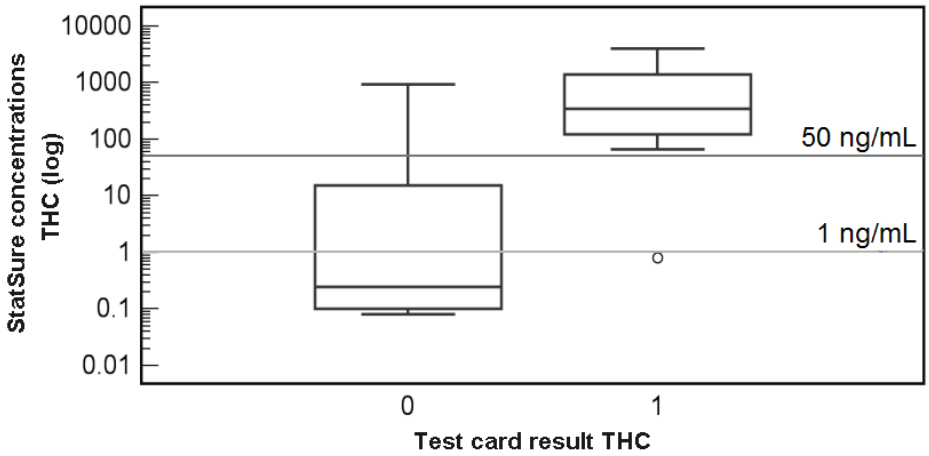


Figure 5: Box-and-whisker plot for THC: StatSure™ concentrations (log) according to test card result (0 = negative, 1 = positive); compared to the Varian cut-off (50 ng/mL) and the DRUID cut-off (1 ng/mL)

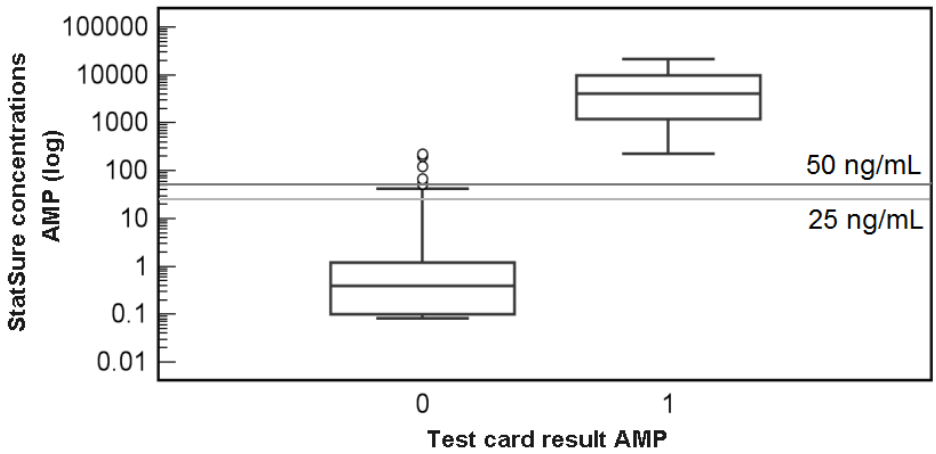


Figure 6: Box-and-whisker plot for amphetamines: StatSure™ concentrations (log) according to test card result (0 = negative, 1 = positive); compared to the Varian cut-off (50 ng/mL) and the DRUID cut-off (25 ng/mL)

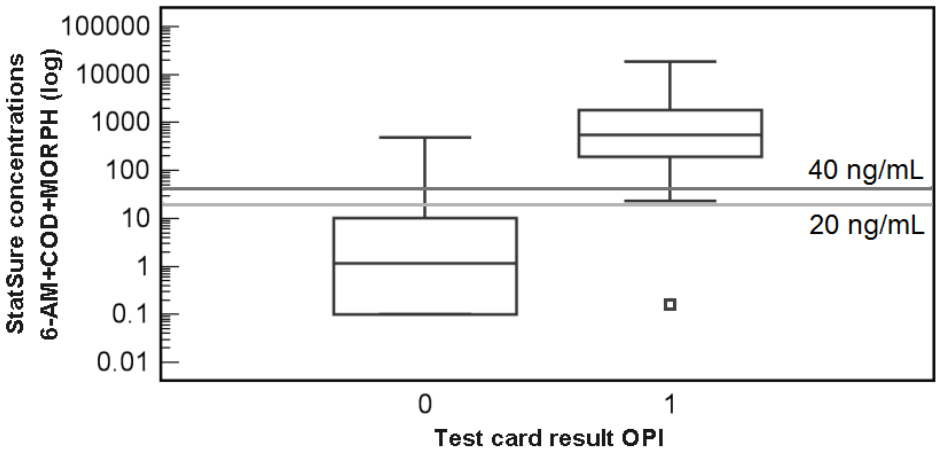


Figure 7: Box-and-whisker plot for opiates (6-acetylmorphine + codeine + morphine): StatSure™ concentrations (log) according to test card result (0 = negative, 1 = positive); compared to the Varian cut-off (40 ng/mL) and the DRUID cut-off (20 ng/mL)

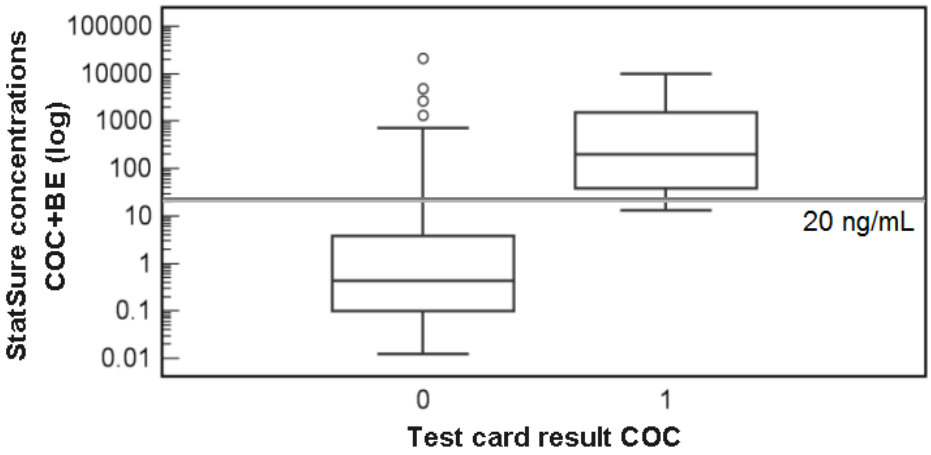


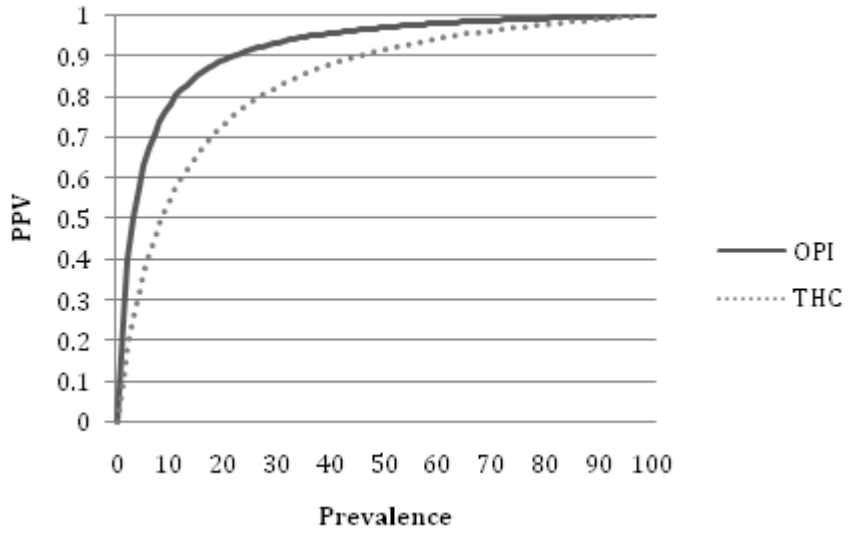
Figure 8: Box-and-whisker plot Cocaine + Benzoylecgonine: StatSure™ concentrations (log) according to test card result (0 = negative, 1 = positive); compared to the Varian and DRUID cut-off (both 20 ng/mL)

Positive and Negative Predictive value

The positive and negative predictive values (PPV and NPV) can indicate whether a test is also applicable in other populations than in the one selected for the study. PPV and NPV are prevalence-dependent, this relation is reflected in the theorem of Bayes and it is important to keep in mind when applying these values on other populations.
In this case it is important to apply the results from the study to traffic settings and police controls, where prevalence values are low.

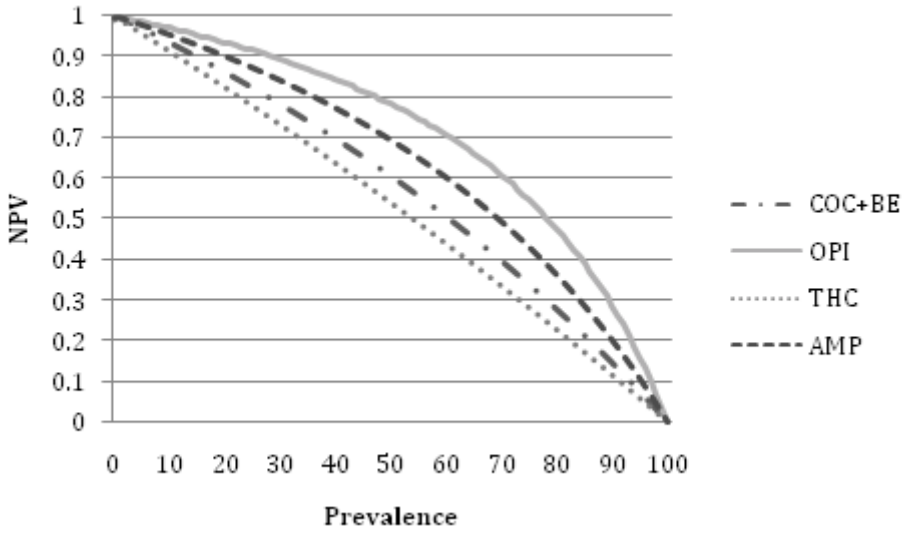
PPV and NPV were calculated for prevalence from 0% till 100% and are displayed per drug type in figures 9 and 10. PPV was 1 for each prevalence value in the case of cocaine and amphetamine, due to a specificity of 100% for those drugs; therefore it is not represented in figure 9.

In traffic, the normal prevalence of drug use is approximately 1%, this gives a PPV of 25% for opiates and 10% for THC, NPV is 99% for all drugs.



OPI = opiates (morphine, codeine and 6-acetylmorphine); THC= delta9-tetrahydrocannabinol

Figure 9: Positive predictive value plotted against prevalence.



COC+BE= cocaine + benzoylecgonine; OPI= opiates (morphine, codeine, 6-acetylmorphine); THC= delta9-tetrahydrocannabinol; AMP= amphetamine

Figure 10: Negative predictive value plotted against prevalence.

Discussion

The prevalence in this study is not the prevalence seen in a traffic setting, since 80% of the study subjects were recruited in a drug rehabilitation centre and only 20% in a normal traffic

setting. Within the 200 study subjects recruited in the drug rehabilitation centre, 85% tested positive with UPLC-MS/MS for one or more drug types. The normal prevalence of drug use in traffic is approximately 1%.

Looking at the prevalence for each drug separately, we see THC and opiates have the highest percentages (~45%) with the DRUID cut-offs. Cannabis is a widely used drug and earlier research showed that THC is the most prevalent drug among impaired drivers in Belgium, followed by amphetamine and ecstasy [11]. In this study opiates follow THC regarding prevalence, but this was also as expected, since the majority of the recruited drug addicts come to the rehabilitation centre for methadone substitution, to control their heroin addiction.
The 50-fold higher Varian cut-off for THC (50 ng/mL) results in a much lower prevalence (only 17%), which indicates that 28% of the THC concentrations were between 1 and 50 ng/mL.

Figures 5 to 8 illustrate that the lowest detectable concentration with the Varian Oralab®6 test card is higher than the one mentioned by the manufacturer. Even with the higher Varian cut-off values, the test card is not sensitive enough for cocaine and THC.
Comparing the results of this study with the results obtained with the Varian OraLab tested during the Rosita-2 project, there are some significant differences. While the OraLab faced a failure percentage of more than 25%, and many officers lost their enthusiasm to continue recruiting subjects, there were no such problems during the study with the Varian Oralab®6. At the other hand, the sensitivity in the present study is lower than in the Rosita-2 evaluation (table 6). This is probably explained by the fact that in Rosita-2 the negative screening results with OraLab were not systematically confirmed, which resulted in an overestimation of the sensitivity.
The sensitivity of the Oralab®6 is also lower than most other tests of Rosita-2. Specificity and accuracy was in general better than seen in those other tests. But the threshold for a sensitive test was 90%, so anyhow, further research on and improvement of the test card of the Varian Oralab®6, definitely for cocaine and THC, is absolutely necessary.

Table 6: Prevalence, sensitivity, specificity and accuracy of the Varian OraLab, tested during the Rosita-2 project [4].

	Prev (%)	**Sens (%)**	**Spec (%)**	**Acc (%)**
COC+BE	19.3	97.2	96.7	96.8

OPI	3.7	100.0	100.0	100.0
THC	13.5	73.9	99.3	95.9
AMP	1.6	66.7	98.4	97.9

Walsh et al. [12] also tested if the cut-off concentrations postulated by the manufacturer were achieved. For most tests, false negatives and false positives were detected for each drug type. The OraLab gave false negatives for opiates and THC and false positives for cocaine. Looking at the results of the Oralab®6 in this study, there are false negatives for each drug type and for all, except for amphetamine, false positives.

Conclusion

The aim of this study was to evaluate the reliability of the Varian Oralab®6 to determine whether this rapid on-site oral fluid test can be used for drug detection during police controls. Applying the DRUID cut-off values, the sensitivity of the test is too low, especially for cocaine (35%) and THC (16%). Specificity was high for all drugs (98-100%).

Acknowledgements

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